R E M A R K S

Claims 245, 247-255, 262, 265, 268-271 are pending in the above-referenced application. As will be discussed in further detail, claims 245, 255, 262, 270 and 271 have been amended to more distinctly claim the subject matter of the invention and to advance prosecution. These claim amendments are supported by the specification on page 86, lines 4-6. Applicants do reserve the right to file subsequent continuation and/or divisional applications on the canceled subject matter.

Furthermore, as will also be discussed in further detail, claim 271 has also been amended to overcome an objection and rejection under 35 USC §112, second paragraph.

1. The Invention

Applicants would like before addressing the rejection, summarize the essential elements of the invention. The invention is directed to a construct comprising a nucleic acid sequence encoding a polymerase (claims 245-251), gene product (claims 262, 265 and 270) and gene product toxic to a prokaryotic cell (claims 255 and 271). In the constructs of the present invention, insertion of an intron will disrupt a coding sequence of a selected protein and splicing out of the intron will return the activity of the protein.

2. The Rejection Under 35 USC §112, First Paragraph (Written Description)

Claims 245, 247-255, 262, 265, 270, and 271 are rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. Several assertions were made in the Office Action. The assertions and responses are set forth below.

On page 3, lines 3-13, the Office Action states:

> Applicant argues that the majority of insertion events would generate frame shift mutations in the coding sequence and inactivate a target gene. Applicant argues that the fact that a frame shift mutation in a transcript will inactivate gene expression is well

> known in the art. It is agreed that the insertion of an intron into a coding sequence may inactivate gene expression. However, as set forth by applicant, 1/3 of the time, such an insertion will not inactivate gene expression. Therefore, the instant specification does not adequately describe insertion of any intron into any sequence encoding any polymerase in a manner that the construct would necessarily result in being incapable of being expressed in a prokaryotic cell and capability of producing more that one copy of a sequence, not even necessarily the same sequence, when introduced into a eukaryotic cell.

In response, Applicants take issue with the word "may" since as admitted in the next paragraph of the Office Action, 2/3 of the time, insertion of intron sequence will put the protein out of frame and as such it indicates that most of the time inactivation should take place. The use of the word "may" seems to imply a more unlikely event that on occasion will take place.

The Office Action in the paragraph bridging pages 3 and 4 and paragraphs on page 4:

> Applicant argues that undue experimentation would not be needed because the expectation of success is 2/3 of the time. However, the claims are extremely broad and do not set forth any criteria for the intron or target sequence other than that nucleic acid encodes a polymerase. The specification does not describe such a huge genus of nucleic acid constructs in a way that one of skill in the art would be able to recognize that applicant was in possession of the claimed genus at the time of filing. The specification does not set forth any specific structural feature to describe the genus of constructs that would result in the claimed activity. Although applicant asserts that it is a readily ascertainable property to choose an intron candidate, the specification has not described what feature of the intron would necessarily result in the instantly recited outcome. Due to the breadth of the instant claims, one of skill in the art would not be able to readily envision the instant genus of constructs that would be incapable of expression in a prokaryote but capable of producing a nucleic acid sequence in a eukaryotic cell, as splicing is not a predictable event with regards to such a broad genus of introns and nucleic acid sequences.

> Applicant asserts that the specification sets forth specific sequence guidance features that are part of the intron sequences on pages 84 and 85, for example that consensus sequences should be present and that perhaps a frame shift mutation and/or stop codon should be present. However, these are not elements of the instant claims and are set forth in the specification as possible embodiments. The specification does not describe such a broad genus as instantly recited in a way that would allow one of skill to envision which species of the instant genus would in fact operate in the claimed fashion, given that splicing events are sequence specific and unpredictable in the art.
>
> Applicant explains that the presences of stop codons and/or frame shift mutations will prevent expression in prokaryotes or eukaryotes and that splicing machinery in eukaryotes allow for expression. However, these elements are not necessary in the instant claims and the specification does not describe nucleic acid constructs commensurate in scope with the instant claims that would allow one to envision the species of possible introns in possible locations in any possible nucleic acid construct that encodes a polymerase that would necessarily be spliced and result in expression in eukaryotes.

In response, as pointed out in previous responses, it was well known in the art as of the priority date of the instant application that one of the characteristics of intron sequences is the presence of stop codons in all three reading frames. As stated on page 386 of "Genes III", a college textbook published by Benjamin Lewin in 1987, "Introns of nuclear genes generally have termination codons in all reading frames…." (submitted herewith and made of record in the Supplemental Information Disclosure Statement also submitted herewith). A subsequent revised edition, "Genes VI" published in 1997 (shortly after the priority date of the present application) contained the same quotation on page 667 (submitted herewith and made of record in the Supplemental Information Disclosure Statement also submitted herewith). As such, it can be seen that a random choice of any published intron sequence will also likely result in disruption of expression due to early termination after an insertion event into a

coding sequence. Armed with the teachings of the present invention as well as knowledge incumbent upon one of skill in the art, it would be a trivial matter for the skilled artisan to select a published intron sequence that would virtually guarantee inactivation of a gene when inserted into a coding sequence.

Applicants do wish to point out that in many instances, insertion of a new sequence into the coding region of a protein is likely in and of itself to eliminate activity even if the insertion retains the same reading frame and contains no stop codons at all, since the presence of the foreign amino acids are very likely to disrupt secondary structures, enzymatic binding sites or enzymatic catalytic sites that are necessary for the protein's function. This system, in general can be referred to as "insertional mutagenesis" and is a historically well known and widely used system for investigating gene functions. For instance, a review of the method was published about the time of the filing in 1996 by Jonkers and Berns (Biochim Biophys Acta 1287; 29-57) (submitted herewith and made of record in the accompanying Information Disclosure Statement also submitted herewith) using retrovirus integrations to disrupt gene functions.

Applicants also note that in response to the assertion that frameshift mutations and/or stop codons "are not elements of the instant claims and are set forth in the specification as possible embodiments", due to the nature of introns, frameshift mutations and/or stop codons are inherently present in most of the introns known in the art as of the priority date of the instant application. Claims 245, 255, 262, 270, and 271 have been amended to recite that the gene product is incapable of being expressed in a prokaryotic cell due to stop codons and/or frameshift mutations introduced by the presence of said intron.

It is stated on the first and second paragraphs of page 5

> Applicant asserts that there are a large number of introns available that will fulfill the properties of the instant claims. However, there is no guidance in the specification that would define this subgenus, whereas the claims embrace insertion of any intron at any location within the nucleic acid construct with resultant splicing in eukaryotes.
>
> Applicant asserts that there is allowed to be complexity in the machinery. The examiner is not

> arguing the level of complexity, but rather is arguing that one would not be able to envision the instant genus of molecules as instantly recited and sets forth art to support the unpredictability of the splicing machinery.

In response, as discussed in previous responses, the sequences of numerous introns were widely published at the time of the filing date of the instant application. One of ordinary skill in the art would certainly not be constrained to selecting a sequence blindly with only the final experimental results available to determine whether an appropriate choice of an intron has been made. As such, even prior to doing a single experiment in a laboratory, the skilled artisan would be able to select and evaluate an intron and evaluate its sequence to determine the presence of stop codons as well as predict effects on frame shifts after an insertion event. As such, even before carrying out a single physical step, the skilled artisan would have the ability of the chosen intron to be able to disrupt a coding sequence by insertion of their selected intron. Thus *contra* to assertions made in the Office Action, one of ordinary skill in the art would be able to envision the genus of molecules as instantly recited.

It is stated in the last paragraph of page 5:

> Applicant points to the SV40 intron, as disclosed in the specification, and explains that the SV40 intron contains stop codons in all three reading frames. Applicant sets forth mathematical calculations on the probabilities of a codon being a stop codon and points to Schwartz et al. for teaching an intron inserted into a coding sequence that resulted in a frame shift mutation. Although there are introns in the art that certainly contain stop codons or would result in a frame shift mutation, the specification does not set forth any specific property that would result in incapability of being expressed in a prokaryote while able to produce copies of a transcript in a eukaryote and it is not evident that insertion of any intron would have these results. It appears that applicant is relying upon the assumption that eukaryotic splicing machinery would necessarily result in splicing of any intron located in any sequence encoding any gene product. The Schwartz et al. reference reports on a

specific intron that would result in two in-frame stop codons as well as a reading frame shift.

In response, Applicants note that in the particular instance of an intron's ability to disrupt function of a target function, requirements for structure-function are well established in that a person skilled in the art has knowledge that if the number of nucleotides of the insert are 3n+1 or 3n+2 rather than 3n, the protein will be out of frame for the coding region downstream from the insertion site. Similarly, the particular nucleotides that make up stop codons in a DNA sequence are known to those of skill in the art as being TAA, TAG and TGA. As such, a person skilled in the art would easily be able to envisage nucleotide sequences that will disrupt a protein by this method as well.

Further, Applicants previously had referenced and made of record Yoshimatsu and Nagawa (1989), Science 244:1346 (hereinafter "Yoshimatsu") as describing the introduction of an intron into a coding sequence. This intron was characterized as both disruptive (white colonies in a lacZ experiment) and yet capable of being spliced out (and thereby producing blue colonies under the proper circumstances in the lacZ experiment). The mechanism by which the inserted intron would disrupt gene activity was never alluded to and the article was written as if the mere description of the presence of an intron was sufficient for readers of the article to understand this disruption. However, an examination of the sequence shown in Figure 1 of Yoshimatsu shows that blunt end ligation of the insert (the SnaB1/Pvu II "cassette") into a site would cause a frame shift mutation (the 102 nucleotide sequence was [n+2] in terms of triplets) and it had stop codons in all three reading frames. Apparently these features were of such an obvious characteristic of an intron that they never needed to be even mentioned to the skilled artisan who would be reading the article. It also should be noted in passing that the insert of the intron described in this since yeast do not need any particular flanking exon sequences to operate function. As such, the blunt ends of the insert are also the ends of the regions of the intron that will be spliced out, thereby reconstituting the normal sequence. Three different sites in the URA3 gene were tested with this cassette and in all cases proper splicing out of the insert was observed.

It is stated in the three paragraphs of page 6 of the Office Action:

> It is important to note that the instant claims are not limited to the embodiments addressed by applicant above. The specification does not provide support for the use of any intron, in any polymerase or any bacteriophage polymerase, or any conditionally toxic gene, in any eukaryotic or prokaryotic cell because the specification provides only minimal description of any particular intron, polymerase (including bacteriophage polymerase), or toxic gene, or eukaryotic or prokaryotic cells for whom known structures exist that could be utilized having the claimed function.
>
> Applicant points to Mount et al. for teachings regarding knowledge of numerous introns. It is agreed that many introns were known in the art, but one of skill would not have been able to envision which ones would act in the context of the instant claims. Regarding "toxic gene", it is acknowledged that the instant specification discusses some examples of a gene being considered toxic in a prokaryotic cell, however the instant specification does not set forth any structural feature that would allow one of skill to envision which genes are considered toxic versus those that are not within the context of the instant claim breadth.
>
> It is acknowledged that it is well known that prokaryotic cells lack splicing machinery that is present in eukaryotic cells. Therefore, the lack of written description is not based upon the differences between prokaryotic and eukaryotic cells, but rather is based upon insertion of any intron into any sequence encoding any polymerase with this resultant action.

Applicants assert that as discussed in previous responses, the ability of stop codon or frameshift mutation to eliminate expression is not dependent upon the choice of a particular polymerase or toxic gene. Thus, the choice of polymerase or toxic gene is not significant. The particular enzymatic function that a protein target carries out is totally irrelevant to the ability of a stop codon or a frameshift mutation to render it non-functional since it is based strictly on the physical properties of the presence of the nucleotides in an intron that will produce frameshifts or stop codons. Universally, it can be said that stop codons

function as their name implies, they stop translation and produce aborted protein sequences. In a similar fashion, a frameshift mutation renders the amino acids translated after the mutation site into a meaningless peptide sequence that lacks any relationship to the normal sequence that would ordinarily ensue. No particular effect on the ability of a stop codon or a frameshift to eliminate functionality is associated with a particular functionality of the target per se. In prior responses, Applicants have made reference to Yoshimatsu, which describe the addition of artificial introns into target genes where in their Abstract they state: "The advantage of this intron-mediated control system is that **any gene** can be converted to a controllable gene by simple insertion of an intron." (emphasis added).

It is asserted that the specific recitation of "prokaryotic" and "eukaryotic" is sufficient in and of itself, since as acknowledged in the Office Action, all procaryotes lack splicing machinery and all eukaryotic cells have splicing machinery. The teachings are adequate to describe the use of an intron which will prevent expression since regardless of its sequence. The intron remains part of the coding sequence of the transcript in any and all prokaryotic cells and adequate teaching is provided to ensure that there is a likely result of having removal of the artificially added intron sequences by the simple expediency of introducing the construct into a eukaryotic cell environment where the resident cellular machinery should be perfectly adequate for removal in a normal and predictable manner, thereby restoring functionality to the target protein. Further, the Mount reference also give multiple references to intron sequences that can be examined for their suitability in the practice of the present invention. Applicants again emphasize that the choice of polymerase or toxic gene is not significant.

It is stated in the paragraph bridging pages 6 and 7 of the Office Action:

> The specification provides for the use of T3, T7 or SP6 polymerases, and also for the use of certain "consensus" splice donor and acceptor sites for inserting introns. Applicants prophetically suggest that intron "insertion at any of these sites in a gene coding region should not affect subsequent removal of the

> processing element in a compatible cell." (page 84 of the instant specification). However, there is significant unpredictability in such intron removal, since such a process requires a complex interaction between the nucleic acid construct and the already existent cellular machinery.

In response, as asserted in previous responses, the insertion of an intron sequence should predictably allow its subsequent removal when present in a eukaryotic cell. In response to the statement in the Office Action that "such a process requires a complex interaction between the nucleic acid construct and the already existent cellular machinery", Applicants note that the interaction takes place between an intron and resident splicing machinery and there are a wide variety of intron sequences that are already known in the literature to be the natural substrates for the cellular splicing processes. Applicants would also like to point out that this level of complexity has not deterred researchers from successfully designing and using artificial introns. This assertion in the Office Action is analogous to a belief that no plasmid can ever be claimed since its replication in a host requires interaction between the nucleic acid construct and already existent cellular machinery used for DNA replication. In a similar vein, claims for expression of a protein from a vector should never be allowed since transcription/translation requires interaction between the nucleic acid construct and the already existent cellular machinery for transcription of RNA and synthesis of proteins by ribosomal complexes, a protein/nuclear complex that is even more complex that the spliceosome. Just the fact that the process required by resident factors of the cell is of a complex nature does not mean that it is unpredictable in its behavior even when the appropriate nucleic acid sequence signals are present.

It is stated on the first paragraph of page 7 of the Office Action:

> Applicant argues that the *(C/A)* AGG sites in the target genes resemble a postsplice site and points to Dibb for support of this concept. Applicant argues that these sites will be converted into splice donor and acceptor sites by the addition of the flanking intron sequence. If the presence of a *(C/A)* AGG site is what applicant is relying upon for the instant mechanism to

> occur, this should be an aspect of the instant claims. Claims 262, 270, and 271 are the only claims that require a *(C/A)* AGG site, but these claims are directed to constructs comprising a nucleic acid sequence encoding any gene product, where the specification does not describe that this mechanism would necessarily occur in any gene with a *(C/A)* AGG site.

In response, Applicants assert that the use of C/AAGG sites is not a required feature but represents a preferred embodiment. It was noted in the specification that intron sequences can successfully be inserted into the target coding sequences even though they may comprise short flanking sequences that will already contain the CAG as described by Mayeda and Oshima, 1990, Nucl. Acids Res. 18: 4671 (hereinafter "Mayeda and Oshima" and previously made of record). They then concluded that "The results presented here show that when the intact intron is flanked by three conserved nucleotides (CAG) of 5' exon, the intron is **always** efficiently spliced out, **regardless of surrounding exon sequences or position**….."(emphasis added). In addition to the information concerning CAG, Applicants wish to again emphasize the expressed opinion of experts in the field that the exon sequences are not normally important factors. The use of cassettes that lacked the CAG at the 5' end were also functional in the Mayeda and Oshima publication but "on the other hand, transcripts carrying mI cassette were spliced with various degrees of efficiency, depending upon the position of the insert." This is an indication that the CAG sequence may not be required for activity *per se*, but rather for optimal activity. As written, the claims encompass the possibility of additional sequences (such as CAG) also being present since comprise language. It also should be pointed out that it is known that one group of eukaryote, yeast, don't require any particular exon sequences flanking the splice borders. Thus C/AAGG sites are not essential features. Even assuming *arguendo* that (C/A)AGG site is required, Applicants assert that the rejection should not apply to claims 262, 270 and 271.

Balvay is again brought up in this Office Action. Specifically the Office Action in the paragraph bridging pages 7 and 8 states:

> Although applicant asserts that splicing is predictable and argues that the Balvay et al. reference, Balvay et al. indicates that the splicing machinery is highly dependent upon recognizing and interacting with such secondary structures in making the splice and therefore demonstrates that there are additional considerations in splicing mechanisms. Balvay et al. indicates that the addition of a secondary structure to an existing mRNA can cause the cell to splice at a point not normally spliced at, while removal of such a structure can cause splicing to be eliminated (for example see pages 165 bridging to 166). Furthermore, Balvay indicates that the exon plays a significant role in splice site recognition by the cellular splicing machinery. Since one of skill would understand that the nucleotides in the exon remain in the mRNA (or ribozyme) after splicing, applicants claimed nucleic acid constructs, following splicing, would likely therefore contain elements of these exon recognition sites. Such unpredictability indicates that the genus of nucleic acid constructs comprising any intron in any polymerase (or any bacteriophage polymerase), or any toxic gene, and that are active or inactive depending on whether they are found in prokaryotic or eukaryotic cells is very large. Regardless of applicant's specific interpretation of the scenarios of Balvay et al., Balvay et al. demonstrates that applicant has not adequately described the instant breadth in a manner that one of skill would be able to readily envision the instant constructs and would not be able to readily envision the specific genus of constructs that would result in the instant outcomes.

In response, Applicants wish to emphasize that Balvay is a *post facto* discussion of splicing events in exceptional cases where splicing did not take place where they were predicted to take place (or as the Office Action summarizes on page 7 "at a point not **normally** spliced at" [emphasis added]). Applicants disagree with the contention on page 8 that "Balvay indicates that the exon plays a significant role in splice site recognition…." A proper assessment in the Applicants view is that Balvay indicates that although the exon does not normally play a significant role in splice recognition, there are exceptional cases

where the nature of exon sequences can be used to explain splicing not taking places at sites that would have been otherwise predicted.

The Office Action states in the paragraph bridging pages 8 and 9:

> Furthermore, applicant asserts that the methods used to block expression are not related to the ultimate function of the protein and therefore the only knowledge necessary would be the sequence of the protein or polymerase so that an appropriate site could be chosen. However, the instant specification does not describe such a broad genus of nucleic acid constructs that would conditionally control the expression of any polymerase or protein sequence based on the presence of any intron in any eukaryotic or prokaryotic cell. The specification does not disclose a structural characteristic that would allow one of ordinary skill to recognize which introns introduced into which sequences would result in expression or lack of expression of which polymerases or proteins. Applicant argues the necessity of a structural characteristic in the claims. However, the instant genus is extremely broad. In order for one of skill to recognize that applicant was in possession of such a huge genus when the art points to unpredictable results within the genus, there is a need for a nexus between the instant claim breadth and the instantly recited activity.

It appears that the supposed breadth of the claims are problematic according to comments on page 9 of the Office Action in view of the statement "when the art points to unpredictable results". As stated above by Applicants, the results are eminently predictable as judged both by prior art examples. As noted above, in the constructs of the present invention, insertion of an intron will disrupt a coding sequence of a selected protein and splicing out of the intron will return the activity of the protein. Methods for selecting intron sequences are known. This is sufficient.

The Office Action states in the only complete paragraph on page 9 and the paragraph bridging pages 9 and 10:

> Contrary to applicant's assertions, the specific example given in the specification is not representative of the broad genus of nucleic acid constructs that are instantly being claimed. The

> structural characteristics recited in the instant claims are extremely broad and the specification does not disclose a structural characteristic that would allow for the skilled artisan to envisage the entire genus claimed of nucleic acid construct with any intron that would result in any polymerase to be incapable of being expressed in any prokaryotic cells and capable of producing a nucleic acid sequence when introduced into any eukaryotic cell. Therefore, the skilled artisan would not be able to recognize that applicant was in possession of such a broad genus of nucleic acid constructs at the time of filing.
>
> Applicant argues that one of skill is fully capable of recognizing the characteristics that would allow a user to choose a particular intron and that SV40 is an example of a wide variety of introns that would be understood to be of use in the present invention. As explained above, one of skill would not be able to readily envision the instant genus of constructs because the claims do not set forth any structural characteristic that would describe which introns inserted into sequences encoding which polymerase would result in the instant activity, as it is acknowledged in the art that there are additional considerations in splicing, as evidenced by Balvay et al., and that the breadth of the instant construct would not necessarily result in the instant outcomes.

Applicants, in response, would disagree that the structural characteristics that would allow the user to recognize the particular utility of an intron for carrying out the intended function could not be readily envisioned by the user. As noted above, the nucleotide identity of a stop codon is a structural characteristic and the sequences of all three possible triplets coding for them are known to the user; likewise the number of nucleotides that would result in a frames shift mutation (N+1 or N+2) are structural characteristics that would also be well known to the user. Similarly, Yoshimatsu gives exemplification of an intron that induced a frame shift mutation after insertion and included stop codons that would prevent expression as well (See sequence in figure 1). This teaching is also available in Gatermann et al., 1989, Mol. Cell. Biol. 9:1526 (hereinafter “Gatermann”, previously made of record) where the intron used for disruption did not induce a frame shift mutation but had two in frame stop codons (See Table 1 sequence).

Schwartz, 1993, Gene 127:233 (hereinafter "Schwartz", previously made of record) also contains this information, where the intron that they used was characterized as "Furthermore, unspliced *neogst3* mRNA would contain two in-frame stop codons as well as a reading frame shift that would be expected to produce a non-functional *neo* gene product." Thus rather relying upon the specification alone, information was publicly available as of the priority date of the instant application on other intron sequences that were shown to be able to block expression after being inserted into a coding sequence.

Reference is made to Jaillon et al. on page 10 of the Office Action. Specifically, it is stated

> Furthermore, Jaillon et al. (Nature, Vol. 451, 2008, pages 359-363) teach that most eukaryotic genes are interrupted by non-coding introns that must be accurately removed from pre-messenger RNAs to produce translatable mRNAs and that the mechanisms specifying the correct sites remain poorly understood. Jaillon et al. teach that short introns recognized by the intron definition mechanism cannot be efficiently predicted solely on the basis of sequence motifs. Jaillon et al. teach that the intrinsic efficiency of splicing varies widely among introns (see abstract).

In response, Applicants note that an important aspect of Jaillon et al. is that it primarily relates to predicting splicing patterns for sequences of unknown nature rather than the present invention where introns that have already been described and characterized are inserted into new locales. There is an immense dichotomy between predicting the sites of splicing reactions where the only information is the sequence itself, and predicting splicing patterns from the introduction of an intron sequence into a new environment where the intron has already been characterized and isolated such that it's properties are known. It is widely understood that the first scenario is very hard to predict while those skilled in the art have no difficulty in making predictions for the second case. Applicants assert that the statement that "Jaillon et al., teach that the intrinsic efficiency of splicing varies widely among introns" is of limited relevance since this is not a comment upon whether the invention would work or not but rather

how efficiently it would work. Thus in regard to the present claims, it would only relate to how much of the proper product would be made after introduction into a eukaryotic cell. Also, it can be noted that Jaillon states on page 359, col. 1 "That such small introns are recognized through intron definition, as in other unicellular eukaryotes[11], is supported by our observation that **introns inserted into the coding sequence of green fluorescent protein are efficiently spliced out** (data not shown)." (emphasis added). Thus the Jaillon references is actually evidence that inserted introns behave properly and predictably.

In view of the above arguments, Applicants assert that the rejection under 35 USC §112, first paragraph (written description) has been overcome. Therefore, Applicants respectfully request that the rejection be withdrawn.

3. The Rejection Under 35 USC §112 (Enablement)

Claims 245, 247-255, 262, 265, 270, and 271 has been rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the enablement requirement. Several assertions were made in the Office Action. The assertions and responses are set forth below.

The Office Action specifically states in the paragraph bridging pages 10 and 11:

> Although applicant asserts that there is sufficient description for choosing intron sequences, there is not sufficient description for choosing intron sequences within the context of the instant invention, as it is not evident that insertion of any intron into any sequence encoding any intron, especially wherein the insertion is at any position, would result in the instantly recited outcomes. The instant claims are not closed to introns with any specific structural characteristic that would narrow the genus to those introns that are predictably spliced in eukaryotes, as discussed above.

Similar assertions are made throughout the rest of page 11.

Applicants dispute these assertions. In Applicants' view, the specification allows the skilled artisan to practice the invention. As stated in other responses, the characterization of "the unpredictable nature" is unfounded and strictly an

extrapolation derived from a publication (Balvay) that does not concern itself with predictions.

It is stated in the Office Action in the paragraph bridging pages 12 and 13:

> Applicant points to Schwartz, Mayeda and Oshima for teachings of instances where introns have been inserted and spliced in eukaryotic cells and not in prokaryotic cells. It is acknowledged that insertion of an intron into a coding sequence may result in splicing of the sequence in eukaryotic cells. However, applicant is not enabled for inserting any intron into a sequence encoding any polymerase or any gene product with a predictable effect of capability of producing more than one copy of a sequence in a eukaryotic cell while being incapable of being expressed in a prokaryotic cell. The results of Mayeda and Oshima are not enabling for a method of inserting any intron into any polymerase or gene product with the instantly recited outcomes. Mayeda and Oshima teach that determinants essential for splicing is localized in the intron itself plus 3 nt of the 5' exon rather than the overall structure of the pre-mRNA. This does not mean that the structure of the pre-mRNA is not important to the slicing process, just that the 3 nt of the 5' exon were more essential. Furthermore, Mayeda and Oshima are considered evidence that determinants/structure of the intron itself is crucial to the process, this supporting that not necessarily any intron would result in the instant outcomes when inserted into a nucleic acid encoding any gene product or polymerase. Furthermore, the 3 nt of the 5' exon were crucial for splicing, wherein instant claim 245, for example, embraces insertion anywhere in any sequence encoding any polymerase with the instantly recited outcomes. Although applicant argues that intron sequences inserted into a target gene at (C/A) AGG sites are likely to be spliced out, instant claim 245, for example, does not require this. Furthermore, Balvay et al. is evidence that the target structure does in fact playa role in splicing, as discussed above.

In response, Applicants take issue with the statement in the Office Action that "This does not mean that the structure of the pre-mRNA is not important to the splicing process, just that the 3 nt of the 5' exon were more important." There

is no evidence that the "3 nt of the 5' exon" were **more** important" than "the structure of the pre-mRNA" since there is absolutely no evidence in Mayeda and Oshima that the structure of pre-mRNA had **any** importance at all in the system they were using. As seen in the paper itself, the Abstract states "…the intron position does not significantly affect the splicing efficiency in vitro", an indication that new secondary structures that may have been formed due to the new environment of the intron did not have any significant effects. Again, the text itself states: "The results presented here show that when the intact intron is flanked by three conserved nucleotides (CAG) of 5' exon, **the intron is always efficiently spliced out, regardless of surrounding exon sequences or positions**, except when the 5' or 3' exon is too short ." (emphasis added) As such, taking the conclusions of Mayeda and Oshima at face value, this reference would justify a conclusion that insertion of an intact intron into a new location would be predicted to be spliced out properly regardless of flanking exon sequences. Applicants do not disagree that the determinants/structure of the intron are important, but do emphasize that for the choice of any particular intron, these determinants/structure are already inherently present such that the properties of the selected intron are known and predictable. With regards to the statement in the Office Action that "Balvay et al., is evidence that there are additional considerations such as secondary structure that would lead to unpredictability, absent evidence to the contrary", Applicants assert that the Balvay reference is not concerned with unpredictable events taking place when an intron is placed in a new environment, but rather a determination of where the intron borders are in its native environment.

The reference, Jaillon, is cited in this rejection as well. Specifically, it is stated on page 14 of the Office Action:

> Although applicant argues that Balvay teaches rare circumstances, Jaillon et al. (Nature, Vol. 451, 2008, pages 359-363) teach that most eukaryotic genes are interrupted by non-coding introns that must be accurately removed from pre-messenger RNAs to produce translatable mRNAs and that the mechanisms specifying the correct sites remain

> poorly understood. Jaillon et al. teach that short introns recognized by the intron definition mechanism cannot be efficiently predicted solely on the basis of sequence motifs. Jaillon et al. teach that the intrinsic efficiency of splicing varies widely among introns (see abstract).

In response, as asserted in response to the Written Description rejection, Jaillon et al. for the most part describes the predictability of determining intron site positions based upon sequence data alone and is not principally concerned with introns outside of their native environment. As noted above, an offhand reference in Jaillon to experiments without data being shown makes note that insertion of short intron sequences behaved predictably with appropriate efficiency.

Further reference is made to other prior art references on the last complete paragraph of page 14 of the Office Action:

> It is noted that introns can be inserted into genes to control the expression of the gene, as evidenced by the state of the art; (including Gatermann; and Yoshimatsu and Nagawa et al., as cited by applicant). However, none of the references are enabling for a broad method of inserting any intron into any position of any sequence encoding any gene product wherein the resultant eukaryotic sequence would express more than one copy of a sequence. None of these references enable the instant genus of predictable splicing of any intron inserted at any position within a sequence encoding any polymerase or gene product.

Applicants disagree. In Applicants' view, the prior art references are enabling support in that they show the use of stop codons and frameshift mutations as physical characteristics of an intron that can cause disruption of gene activity when the intron is inserted into a coding region.

Balvay is again referred to in the paragraph bridging page 14 and 15 and the first complete paragraph on page 15:

> Again, the issue is not whether it was known in the art how to insert introns, but rather how to insert introns in a predictable fashion in accordance with the breadth of the instant claims and have the desired outcome specific to eukaryotic and prokaryotic cells

> with regards to any polymerase, as recited in the instant claims. Balvay et al. is simply an example that secondary structure is one complexity when considering splicing mechanisms. The instant claims embrace insertion into locations such as those taught by Balvay.
>
> Applicant argues that Balvay is contrary to published material that explicitly states that such a procedure is predictable and essentially problem-free. However, applicant has not pointed to any publication or teaching in the art that teaches that the instant claim scope is predictable or problem-free to support this statement.

Applicants disagree with the assertions made with respect to Balvay. Specifically, Balvay does not teach inserting <u>any</u> sequences, and only provides a discussion of splicing events from introns in their native sequence environments. Further, Balvay also does not teach the use of any genes that are either polymerases or are of a toxic nature. Balvay finally in Applicants' view, has no utility as being predictive in terms of the present invention, since it is only a post-facto discussion of <u>unexpected</u> and <u>unusual</u> results when looking at sequences and trying to predict the sites of native introns.

It is stated in the first paragraph on page 16:

> In particular, it is demonstrated that the complex secondary structures of nucleic acids are responsible for their intron excision activity, and furthermore, that predicting the ability of the cellular splicing machinery to splice out precise intervening sequences from disrupted sequences with variable secondary structures such that native activity is restored is considered unpredictable, because the splicing machinery is sensitive to the presence or absence of such structures.

Applicants disagree and assert that there is prior art that would be sufficiently enabling. Specifically, the Schwartz, Mayeda and Oshima references that were discussed on page 12 of the present Office Action are eminently suitable. They all discuss features that are appropriate for blocking expression of genes of interest in prokaryotic environments and their subsequent normal expression in eukaryotic environments. In addition, Applicants have previously

referenced Gatermann who introduced a synthetic 36 base pair oligo (comprising the 5' GT…..AG 3' consensus sequence) in various locations into the ura4 gene where it acted efficiently in carrying out splicing. As summarized in their abstract: "This suggests that the proper signals within an intron are sufficient to initiate and complete a sequencing event **independent of the location of the intron** in the gene." (emphasis added). This is stated later in the text as "These experiments also demonstrate that sequences containing the proper 5' and 3' splicing signals are recognized and spliced out correctly **independent of the surrounding sequence**." (emphasis added). Further, the Yoshimatsu reference noted that any and all restriction sites that created blunt ends would be useful in yeast, since there is no particular sequence in flanking exons that are required for splicing to take place. As stated in the abstract, "The advantage of this intron-mediated control system is that any gene can be converted to a controllable gene by simple insertion of an intron".

It is concluded in this rejection on page 16 that

> In order to practice the invention using the specification and the state of the prior art as outlined above, the quantity of experimentation required to practice the invention as claimed would therefore require the *de novo* determination of intervening sequences that can be fully spliced out without leaving behind any nucleotides that might interfere with native activity. In the absence of sufficient guidance from the specification, the amount of experimentation would be undue, and one would have been unable to practice the invention over the scope claimed.
>
> Applicant argues that there is no need for *de novo* determination of intervening sequences because the art teaches introns that can be fully spliced out. The examiner is not arguing that there is not evidence of intron splicing in the art, but rather lack of evidence of predictable splicing and expression in eukaryotic cells commensurate in scope with the instant claims.

Applicants again disagree. There is certainly adequate evidence of predictable splicing and expression in eukaryotic cells. The Office Action states that the method would require undue experimentation since it would "require *de*

novo determination of intervening sequences that can be fully spliced out without leaving behind any nucleotides that might occur with native activity." Applicants believe that there is no such undue experimentation required since the intron sequences described in the prior art can be used as inserts and we believe that even though it wasn't tested, the intron described in the Examples section is likely to be successful both in terms of being able to disrupt a gene expressed in a prokaryotic environment and to be spliced out correctly to regenerate the original coding sequence. In the second place we believe that experiments carried out after the filing that we have cited demonstrate that even a arbitrary choice of an intron sequence that has been previously characterized in the literature is likely to be successful simply by inserted it into a coding sequence.

As mentioned in previous response and previously made of record, a paper was published by Johansen 1996 (Proc Nat Acad Sci USA 93 12,400-12,405) (hereinafter "Johansen"). Additionally, as noted before, success was seen for various combinations of five different sites and three different introns. It is of particular interest to not that the sites chosen by Johansen were for insertion of an intron were between CAG and G of a CAAG sequence. As noted above, this simple criterion was sufficient for success in Johansen's experiments. Consequently, it was stated that "It is proposed that intron insertion can be used to facilitate manipulation and amplification of cloned DNA fragments that are unstable in, or toxic to E. coli" and then summarized in Johansen's paper as "This strategy could potentially be used to facilitate manipulation of **<u>any</u>** DNA sequence from which toxic products are expressed in *E. coli* from cryptic promoters and initiation codons (emphasis added). Applicants note that Figure 1 of Lopez-Moya et al., 2000 (Virus Research 68; 99-107) (hereinafter "Lopez-Moya et al." and (submitted herewith and made of record in the Supplemental Information Disclosure Statement also submitted herewith) show insertion of an intron between CAG and G of a CAAG sequence; successful elimination by the native splicing machinery of target cells were shown by "Direct sequencing of the amplified fragment confirmed that the splicing had been correctly achieved *in vivo*.....". As Lopez-Moya et al. later noted in their discussion: "Intron insertion in

viral genomes (Johansen, 1996) is an **easy** alternative to deal with cloning problems and we used this approach with success." (emphasis added).

The C/AAGG consensus proto-splice sequence has been used subsequently by others after the present filing. This work verifies the lack of problems in adding intron sites previously lacking introns. These papers that are admittedly post-filing are not meant to add credence that the specification is enabling, but rather to counter the arguments that the Balvay reference renders the present method unpredictable and as such requiring undue experimentation. For instance, Bensaad et al., 2003 (J Biol Chem 278; 10,546-10,555 and submitted herewith and made of record in the Supplemental Information Disclosure Statement also submitted herewith) devised an artificial gene with "artificial introns" dividing 5 domains (exons) of the p53 gene for the purpose of "swapping exons" to investigate functionality. In this publication, they noted "We first verified that the p53 expressed by these minigenes did not contain any alteration because of aberrant splicing". This test confirmed that that their constructs carried out the appropriate splicing regardless of the nature of the insert sites used. In Gonzalez et al., 2002 (J Vir 76; 4655-4661 and (submitted herewith and made of record in the Supplemental Information Disclosure Statement also submitted herewith) introns were artificially introduced into a toxic region of gastroenteritis coronavirus for growth in *E. coli*. As stated in the abstract, "...the intron was efficiently removed during translocation of this RRBA to the cytoplasm" indicating that in the eukaryotic cell restoration of the normal sequence was achieved. It should also be noted that two different positions were tested with eventual success in each case. Bissonette et al., 2006 (Gene 372; 142-152 and (submitted herewith and made of record in the Supplemental Information Disclosure Statement also submitted herewith) shows that nucleic acids coding for antibacterial gene products could be grown in bacterial hosts by insertion of an intron into the coding sequence. As stated in their abstract: "We have found one intervening sequence, which derives from adenovirus, can be spliced in a mammalian system **without respect to its location**." (emphasis added). Another paper that successfully used intron insertion to control

expression of toxic genes in bacteria was published by Ulper et al., 2008 (J Vir Methods 148; 265-270 and (submitted herewith and made of record in the Supplemental Information Disclosure Statement also submitted herewith) where in conclusion they stated "The virus stock collected from transfected cells did not contain detectable amounts of non-spliced or incorrectly spliced RNAs…." showing that appropriate splicing out of the intron took place after the nucleic acids were expressd in mammalian cells.

As such, Applicants assert that a prediction supported Balvay is not supported by various papers that have found insertion of an intron to be reliable in terms of both disrupting the intended gene target and totally predictable in being able to splice out the added sequences in a eukaryotic cell.

In view of the above arguments, Applicants assert that the rejections under 35 USC §112, First paragraph (lack of enablement) has been overcome. Therefore, Applicants respectfully request that the rejection be withdrawn.

4. Double Patenting

Claims 245,247-255,262,265,270, and 271 have been provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1 and 2 of co-pending Application No. 11/929,055. Applicants note that this is a provisional rejection. Applicants will address this rejection upon indication of allowable subject matter.

5. Claim Objections

Claim 271 is objected to because of the following informalities. It is asserted that in claim 271, it appears that applicant inadvertently inserted two commas after the word "intron". In response, claim 271 has been amended to delete that extra comma. Thus, the objection has been overcome and should be withdrawn.

6. **The Rejection Under 35 USC §112, Second Paragraph**

Claim 271 has been rejected under 35 U.S.C. §112, second paragraph, as

being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Office Action states

> Claim 271 recites the limitation "said intron", although the claim does not previously refer to an intron. Therefore, there is insufficient antecedent basis for this limitation in the claim.

In response claim 271 has been amended to recite “an intron is removed…”. Thus the rejection under 35 USC §112, second paragraph has been overcome and should be withdrawn.

7. Conclusion

It is Applicants belief that the pending claims are in condition for allowance. However, if a telephone conversation would further the prosecution of the present application, Applicants’ undersigned attorney request that he be contacted at the number provided below.

Respectfully submitted,

/Cheryl H Agris/

Dated: December 22, 2009

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